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SPORE FORMATION IN PHILOCOPRA COERULEOTECTA

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The greater part of the work done on spore formation in the Ascomycetes has been carried on in connection with the few-spored asci. The many-spored forms have received little attention due to the difficulties attending the smaller size of the spores in most cases. The process of delimitation of spores in a many-spored ascus is of special interest in that it may throw some light on the true nature of the ascus. The variations from the method found in the few-spored forms call for further study.

The review by Sands (24) of the literature on the process of spore delimitation in the Ascomycetes, as well as the monograph by Guilliermond (11) on the cytology of the Fungi, makes it unnecessary to give a detailed account of the work done. A brief review will suffice to show the present status of the question as well as the problems at hand.

The process of spore delimitation in the Ascomycetes was first described by Harper (14). He found that the spores were cut out of the cytoplasm by the bending back of the astral rays and their subsequent fusion to form an ellipsoidal membrane. Harper (12, 13, 14, 15, 16, 17) has described the process in a number of different genera. His work has been corroborated by work on additional forms by Guilliermond (8), Maire (21), Sands (24), the writer (18), and others. This view is opposed by Faull (1) who believes that the membrane is formed at the expense of a granular zone of cytoplasm. He believes the astral rays play no part in the delimitation of the spores.

Harper's view that the astral rays are of the nature of cilia is opposed by Fraser (3, 4), who agrees with him that the spores are delimited by the astral rays in the forms studied by her. Following the hypothesis suggested by Harper (14, page 274)—that the centrosome may be the seat of fermentative activity—Fraser holds the view that at the close of the third division the centrosome may generate a ferment, which, as the nucleus pushes out toward the wall, flows back in its wake, producing a chemical change in the area through which it is distributed. However, there can be no question from the figures described for Geoglossum by the writer (18) that the rays actually bend down toward the nucleus in order to form the membrane. The long duration of the period when the interastral zone is formed by the rays, which run out radially from the center in all directions, renders Fraser's theory quite untenable.

Overton (22) has described spore formation in *Thecotheus pelletieri*, a thirty-two-spored form. He found it to be similar in general to that described by Harper for the few-spored forms. In *Thecotheus* thirty-two nuclei were formed before delimitation. Ramlow (23) reports a similar method of spore formation in *Thelebolus*. The spores are delimited after many nuclei are formed.

Lewis (19) studied the development of the ascus in *Pleurago zygospora*, a sixteen-spored form. The nuclei in this case undergo the usual three divisions, producing eight nuclei, after which delimitation occurs. The spore then elongates to form a filament which subsequently divides to form two spores.

While carrying on some experiments on the light reactions of *Pilobolus crystallinus* at the University of Wisconsin, it was noted that after a culture had been in use for several days, there appeared on the glass used in the experiment some black masses much smaller than the sporangium of Pilobolus. A further examination revealed the fact that these spots contained the spores from a Pyrenomycete. At first this fungus covered the surface with delicate hyphae, which increased until the culture was coated with a white mat of mycelia. Gradually there developed from this mycelium many black flask-shaped fruiting bodies. At first these appeared as little black specks on the white mycelium, but later they covered it in great numbers. The Pilobolus died out as this Pyrenomycete grew. In connection with the work on Pilobolus, which demanded that the glass on which the spores were found be placed at varying distances from the culture, it was noted that this fungus, as well as Pilobolus, could throw its spores a considerable distance and that they were discharged toward an illuminated spot.

The asci from the pyrenocarp contain many spores. It was found that they did not agree with the description given for any known species. The material was sent to Dr. Rehm for identification. Rehm described it as a new species and gave it the name *Philocopra coeruleotecta* Rehm.

The asci were of fairly good size and appeared favorable for the study of their development and the formation of the ascospores in a many-spored form. The many-spored forms are looked to for evidence on the question whether the ascus is in any way related to the sporangium found in the Phycomycetes. The process of delimitation in a many-spored ascus; the stage when delimitation occurs; the presence of a central body; and the relation of the epiplasm and spore-plasm are subjects of considerable interest in the study of the relationships of the Ascomycetes.

The material for this study was fixed in Flemming's weaker solution and Strasburger's modification of Flemming's medium. Both afforded excellent fixation. The sections were stained with modifications of Flemming's triple stain and Heidenhain's iron-haematoxylin.

There was no trouble in getting an abundance of the material in all stages of development. The fruiting bodies, together with the adjacent mycelium, were fixed in large quantities. The substratum was included in many cases in order to get all stages. It proved to be very easily sectioned. There was considerable variation in the stages of development among the different asci in a pyrenocarp. Sometimes mature spores and very young asci were found in the same fruiting body. There were always at least several stages to be found until the pyrenocarp reached its full size.

The pyrenocarp is flask-shaped. The wall is composed of several layers of closely packed hyphae, the walls of which stain darkly. From its base arise a number of asci among which are seen numerous paraphyses. Often the asci in a single pyrenocarp are in various stages of development. There is considerable variation in the number of asci to be seen in a pyrenocarp, depending somewhat on its stage of development. Figure 1 represents a longitudinal section through a pyrenocarp. It shows the typical flask shape with the opening in the neck at the apex. This section shows three immature asci with numerous ascospores. Often as many as eight or ten asci may be seen in a single pyrenocarp with the spores at different stages, affording a good opportunity to trace the progressive steps in their development. Younger ascocarps are, however, more favorable for the study of the young stages before spore formation begins.

In figure 2 is shown a very young ascus. It was located very near the base of the pyrenocarp. The rounded upper portion contains one large nucleus with a distinct nucleole. There is present a clear staining central body, which in this case is opposite the nucleole. The chromatin is distributed in a fine network and shows more or less orientation with regard to the central body, *i. e.*, the chromatin granules run inward from the center in more or less distinct rows. The cytoplasm is comparatively dense at this stage. This stage is only of short duration as the ascus elongates rapidly, taking the more slender form seen in figure 3.

Figure 3 represents a young ascus, which is very slender and cylindrical in shape. At this time the nucleus is usually situated in the central portion of the ascus, in this case being nearer the lower end. The cytoplasm is rather uniformly distributed and appears to have a fine granular structure. A few radiations in the cytoplasm extend outward from the nucleus. The nucleole is not visible in this section. The chromatin is in fairly large masses for the most part, and some connection is visible between those particles.

The ascus undergoes a rapid increase in size, so that when the first division of the nucleus occurs it appears to have become several times its original size. The first division of the primary nucleus is shown in figure 4. The cytoplasmic mass is very great compared with that of the nucleus. The cytoplasm in the upper part of the ascus is very similar to that shown in figure 3. The cytoplasm of the lower portion appears a little more vacuolate. The spindle figure is somewhat curved and very distinct and has a small but clearly staining central body at each end. The chromosomes are few in number. They are short and stain heavily. Fibers extend out from the centers but they are not at all distinct between the plasma membrane of the ascus and the centers in this figure. However, there is a very noticeable bulging out of the membrane opposite the central bodies which may indicate some relation between the membrane and the division figure, such as the radial arrangement of astral rays extending outward from the central bodies. Fibers may be seen extending upward and downward from the central bodies. Those below the spindle were especially distinct and in the plane at which figure 4 was drawn they appear to converge at a considerable distance from the central bodies, making the astral rays appear considerably longer than those of the spindle. The division figure lies crosswise of the ascus.

The nuclear divisions in the ascus follow one another rapidly. The daughter nuclei divide while still in close proximity to each other.

Thus the nuclei occupy the part of the ascus nearest the place where the division of the primary nucleus occurred. The daughter nuclei in each case are completely formed before entering upon a new division. The spindle is intranuclear at first. Often the nuclear cavity remains distinct until the early anaphase.

Figure 5 shows two of the resting nuclei in the four-nucleated stage of the ascus. In this case the ascus was cut obliquely. The nuclei are fairly large and show much the same structure as was shown in the primary nucleus of the ascus. The nucleole and central body are distinct. The chromatin network is typical of that formed in the few-spored Ascomycetes and shows some orientation with respect to the central body. It has a lighter and a darker staining portion, the darker part forming lumps scattered along the finer light-staining thread-like portion. The cytoplasm is somewhat denser near the nuclei but there is no well-marked spore-plasm.

Figure 6 represents a dividing nucleus of the third division. One complete figure is shown which runs almost parallel to the length of the ascus. The spindle is slender. The chromosomes have divided and are passing toward their respective poles, where there is a small distinctly staining central body. The nuclear cavity is still apparent. There is here an interesting arrangement of the cytoplasm with regard to the central bodies. It is very dense in the immediate neighborhood of the centers, from which distinct radiations extend outward in all directions. To the right of the lower central body there is visible an oblique section through the polar end of a second spindle figure. The polar aster is apparent. The cytoplasm is fine with radiations running outward in all directions. They are very fine and dense in the immediate neighborhood of the central body.

In the eight-nucleate stage (fig. 7) the ascus has grown somewhat larger. The nuclei are close together in the region near where the primary nucleus was located. The cytoplasm is denser immediately surrounding the nuclei and more vacuolate in that further remote. The less uniform appearance of the cytoplasm is conspicuous in comparison with the earlier stages. The nuclei appear to be in a normal resting condition. They are much smaller than the primary nucleus. Each nucleus contains a distinct nucleolus. The chromatin appears in fine granules. The central body is visible in the best stained specimens, but is not always apparent. Only three of the eight nuclei are visible in the section.

Figure 8 represents a section of the ascus containing one spindle figure of the fourth nuclear division in the ascus. It is typical of all of the spindle figures present. The spindle with the chromosomes in the center stains densely. The nuclear cavity is still distinct. The central bodies at the poles of the spindle stand out clearly. Numerous delicate fibers radiate out from the central body. These are very long and remarkably distinct which is characteristic of the well-stained figures. The fibers, however, cannot be seen as such except when they lie in the plane of the section. They are extremely delicate, but the large numbers seen in the plane of this figure render them rather conspicuous.

In the sixteen-nucleate stage the nuclei in their successive divisions have gradually scattered through a larger part of the ascus, with the upper and lower portions, however, still containing only cytoplasm. The region of dense cytoplasm surrounding the nuclei is even more distinct than in the earlier divisions. From these denser regions strands of cytoplasm radiate outward in different directions. Figure 9 represents a longitudinal section of the ascus at the sixteen-nucleate stage. Eight nuclei are visible in the section. At this stage the nuclei appear much the same as after the previous divisions, except that they are smaller. The nuclei do not increase much in size after dividing. Their structure is very similar and they are not beaked.

The nuclei continue to divide rapidly until there are approximately 128 in number. The later divisions are essentially like the earlier ones. The spindles are small and with distinct centers at each pole. The astral rays, which are strongly developed, radiate out from the centers. These divisions take place near the periphery of the ascus. Figure 10 represents a portion of the ascus containing a nucleus in the equatorial plate stage. This is one of the late divisions. The spindle is very small but clear, with a distinct central body at each end. The chromosomes are plainly differentiated in the center of the spindle. The nuclear cavity is still apparent. The clearness with which this figure shows up in so small a nucleus is remarkable. The asters are present at the poles of the spindle. The astral rays can be seen extending outward from the central bodies into the cytoplasm. The fibers are extremely delicate, but apparent. The dense fine granular appearance of the remaining cytoplasm near the central body is probably due to the fact that numerous fibers have been cut in sectioning.

At the close of the last division the nuclei are situated near the wall of the ascus. They are very small but distinctly beaked. The central body is at the tip of the beak and appears to be in contact with the plasma membrane of the ascus. The nucleole may be situated at the end of the nucleus farthest from the central body, near the base of the beak, or on any one side. The chromatin is fine and granular. There is a dense region of cytoplasm around the nucleus. The cytoplasm in the center of the ascus is very vacuolate and apparently devoid of nuclei. The nuclei are conspicuously located near the wall. Figure 11 represents a longitudinal section through an ascus at this stage. The central part contains many vacuoles. The nuclei are peripherally located and surrounded by a dense cytoplasm. The beaks are visible on only four of the nuclei figured. The others have been cut across below the beaks in sectioning.

Figure 12 represents a cross-section of an ascus at the same stage. The peripheral arrangement of the beaked nuclei is even more manifest in this view. The large internal portion and the basal and apical regions of the ascus contain only cytoplasm. The centers are distinct and the nuclei are conspicuously beaked. The nuclei are very small at this stage.

Figure 13 represents a slightly later stage. The section has been cut somewhat obliquely from the side of an ascus. It shows only a small part of the ascus near the periphery. The nuclei with their long slender beaks are always pointing toward the membrane, the centers apparently in contact with the latter, although the nuclei in this stage, as well as in that represented by figures 11 and 12, always have the centers in contact with the plasma membrane of the ascus. They are comparatively small at this stage, but the fact that so many of them lie with their longitudinal axis in the plane of the section allows them to be easily observed. There is no definite arrangement as regards their exact direction. This is especially clear from figure 13. The fine cytoplasmic masses surrounding the nuclei are very conspicuous in this figure. Often little fibers can be seen radiating from the centers. A close study of the two beaked nuclei on the left of the figure shows the centers against the plasma membrane with an indentation of the membrane between as if the astral rays of the two nuclei meet in this region. On the other sides of these nuclei there is a similar appearance, reminding one of the stages in *Geoglossum glabrum* Pers. when the interastral zones are formed by the meeting of the fibers of adjacent asters. The lower portion of figure 13 is nearer the periphery. Three or four dense masses of cytoplasm have been cut across. They appear rather closely approximated when seen from this view. The appearance here in general is characteristic of the stage when the interastral region is formed by the meeting of the rays from the adjacent centers prior to the delimitation of the spores. This stage is frequently met with and must be of comparatively long duration.

The spores are delimited while the nuclear beaks point toward the membrane. At the time of delimitation the spore is practically spherical in shape. The membrane is very delicate at first. The cytoplasm contained within the spores is finely granular and dense. The epiplasm—the portion of the cytoplasm outside of the spores—is very vacuolate, except at the periphery of the ascus. The centrosome is conspicuous and stains red. Figure 14 represents a slightly oblique cross-section through an ascus soon after the spores are delimited. The spores are still peripherally located with the centers pointing toward the plasma membrane of the ascus, but not touching it. The membrane of the ascus is intact. The central portion of the epiplasm is vacuolate with delicate thread-like strands of cytoplasm throughout. The nuclei with their beaks are in contact with the spore membrane. The nucleole and the chromatin granules are visible in the wider portion. The center is distinct.

The ascus continues to grow larger. The spores rotate soon after delimitation so that the central body points downward and outward. The beaked nuclei are still in contact with the spore membrane. Figure 15 shows this condition. The ascus is cut longitudinally. The section represented is near the periphery of the ascus. The centers are pointing outward and toward the base of the ascus. The membrane is distinct. The epiplasm is vacuolate throughout the part containing the spores. Clearly defined strands run between the spores and out to the peripheral layer in the ascus. The spores then become much elongated. At the same time the nucleus leaves the spore membrane. The central body is in contact with the nuclear membrane. The nucleus becomes more or less ellipsoidal with the central body toward the lower end and the nucleole at the opposite end. The membrane of the spore is still very delicate. The cytoplasm is less dense than in the earlier stages after delimitation. This condition is shown in figure 16, which represents a small part of an oblique longitudinal section through the ascus. It was drawn especially to show the nuclei at different distances from the lower end of the spore. The spores vary considerably in this respect. In some the nucleus has just left the lower end; in others the nucleus has almost reached the upper end. This difference is not due to the fact that parts of the spores are cut off. A comparison of the size of the different spores will help to make this clear. The spores have elongated and are cigar-shaped. The nuclei are ellipsoidal in form. The nucleole is on the side, usually nearest the upper end, while the center is at the opposite end, thus showing the same relative position as seen earlier. The chromatin is visible in irregular granules of varying sizes. At this stage the upper ends of the spores extend inward to the central portion of the ascus, often giving the appearance of overlapping from the upper end downward. The spores are separated from one another by a small amount of epiplasm. The epiplasm in the upper and lower end of the ascus still takes up a large portion of the ascus, the spores occupying the central portion between. The plasma membrane is still intact.

As the spores increase in size the ascus grows both in diameter and in length. The spores fill a comparatively large portion of the ascus. As the nuclei reach the upper part of the spores, the spores increase in length. At this stage the nuclei are near the center of the spores where the latter are slightly narrower than at either end (fig. 17). The epiplasm in the upper part of the ascus is shown in this figure. It is still finely granular as in the earlier stages. That in the lower portion is much more vacuolate.

The nucleus moves nearer the upper part of the spore, which increases in diameter. The spore is then spherical with a long tail. The nucleus is found in the spherical portion near the place where it connects with the tail. Figures 18 and 19 respectively show these stages in development. The nuclei are spherical in shape and are oriented in various ways. The nucleole and chromatin are distinct. The center is visible in some of the well-stained preparations. The vacuolate epiplasm surrounding the spores is unchanged. The intimate contact of the epiplasm and the spores is marked in figures 18 and 19.

The upper spherical portion of the spore grows rapidly. The nucleus moves toward the center of the spherical part. Figure 20 shows a section at this stage. The cytoplasm is more vacuolate.

After considerable increase in size there is laid down a thick black wall around the upper portion of the spore which is ovoid in shape. The lower tail portion remains hyaline. It is separated from the larger portion by the thick black wall. The cytoplasm in the upper portion stains darkly, while that in the tail portion stains lightly. Figure 21 shows four spores from an ascus after the spore walls are thickened. The spores appear to be mature. The tail is entirely separated from the rest of the spore by a heavy wall. The nucleus occupies the central portion of the spore. The cytoplasm contains some large vacuoles. The tails are hyaline and extremely inconspicuous in comparison with the heavy black spore walls. The cytoplasm stains very lightly. Often near the distal end of the tail in a hyaline spot are found some dense red staining granules. The epiplasm, though comparatively inconspicuous at this stage, is still present, and the plasma membrane of the ascus is intact.

Discussion

The abundance of well-fixed material of *Philocopra coeruleotecta* has made possible a detailed study of the processes in the development of the ascus and spore formation. It has proven especially favorable because of the variety of stages found in a single pyrenocarp.

The nucleo-cytoplasmic relation shows considerable variation. The very young ascus containing the primary nucleus is not at all striking in this respect. The nucleus and cytoplasm do not exhibit any unusual proportion in respect to each other. Immediately following this stage there is a rapid growth in the size of the ascus. The cytoplasm is slightly more vacuolate and has greatly increased in volume. The primary nucleus shows no signs of division for a considerable period. The cytoplasm actually doubles in volume several times while the primary nucleus remains in a resting condition. That part in the immediate neighborhood of the nucleus, however, shows no marked change. Then with further increase in the size of the ascus the primary nucleus divides. A period of successive divisions of the nuclei follows. The divisions follow one another with great rapidity, filling up the central peripheral portion of the ascus. Whether or not there is any relation between this series of rapid successive divisions and the great volume of cytoplasm is a question of considerable interest. If there is a definite relation between the volume of the nucleus and that of the cytoplasm, the quick successive divisions of the nucleus may serve to bring about a balance of conditions in this respect. Even at the close of the series of divisions there is a comparatively large proportion of cytoplasm as compared with the volume of the nuclei in the cell, but this is soon remedied by the delimitation of the spores, which brings about a relation between the two which appears more balanced from our general observations on nuclei.

It is apparent from the figures of the nuclei at various stages in the development of the ascus that the nuclei, although entering on an apparently resting stage at the close of each successive division, do not undergo a period of growth sufficient to bring them to the size of the resting mother nucleus in any case. With every increase in number, there is a decrease in the size of the nuclei. The contents appear to be similar, but each part is proportionally smaller in size. The divisions apparently follow one another so rapidly that there is no chance for much increase in size before a new division intervenes. It is interesting that the primary nucleus of the ascus remained in a resting condition while the cytoplasm increased to such an extent. This condition may be in some way related to the formation of kinoplasmic substance. The close of the division period leaves the ascus with abundant kinoplasm, most of that of the astral rays going toward the formation of the spore membranes.

There is no marked differentiation of spore-plasm in this form such as is found in very many of the Pezizas. Although the nuclei are located in a definite part of the ascus, the general cytoplasm of that region is not distinctly different from that of the remainder of the ascus. That part of the cytoplasm which immediately surrounds the nucleus does not show a remarkable increase during the rapid growth of the ascus up to the delimitation of the spores. Its dense finely granular appearance is apparent in the various stages of development. Most of this region is included within the spore after its delimitation.

The intimate relation between the epiplasm and the spores is conspicuous. It is probable that the epiplasm plays a very significant rôle in the feeding of the spores during their rapid growth after delimitation. The adjacent spores are not crowded together. They have a definite relation to each other and are surrounded by epiplasm. Whether or not the epiplasm plays any other part has not been determined. It would be interesting to learn whether it is concerned with the interrelation of the spores. It undoubtedly plays a part in their final extrusion from the ascus. The long period of existence of such a large amount of epiplasm without nuclei is at least suggestive.

It is certain from the study of *Philocopra coeruleotecta* that the spore wall is laid down by deposition from the outer layer of the cytoplasm within the spore and not by the epiplasm. The spore wall is as thick between the hyaline tail and the main portion of the spore as in the rest of the spore, where it is in contact with the epiplasm. The cytoplasm of the spore has doubtless laid down that portion of the spore wall and probably the remaining part.

The study of the stages in the development of the ascus and in spore formation shows the central body to be present at every stage. The sections were not always stained to bring out a well-defined central body, but in the most favorable preparations the central body stained differently from the chromatin material. It is very small but well defined. In the resting nuclei it appears as a small deep red-staining body in contact with the nuclear membrane. As a rule it is to be found on the side of the nucleus away from the nucleole. The central body is especially well defined in the division figures where the chromatin particles are more remote from it. They are found at the poles of the spindle on the sides of the nuclear cavity. The central bodies are so apparent that there is no doubt as to their existence.

The definiteness of the spindle with the central bodies, even in the late divisions, is too remarkable to leave unmentioned. The minute figures are as clear and distinct as the larger figures. This suggests the possibility of finding more clearly defined figures in some of the other fungi, where they are not sufficiently distinct in the preparations so far figured.

The astral rays can be seen radiating outward from the centers into the cytoplasm in different directions. Some of the preparations are not so favorable as others, but in good clear preparations the rays stand out distinctly. They are very fine, delicate, and numerous. In the fourth nuclear division (fig. 8) a large number of rays are seen in the plane in which the figure is drawn. Figures 6 and 10 show clearly defined asters at the poles of the spindle. Not so many rays are seen in the plane of the figures, but the asters at the poles are unmistakable. There are a number of rays present and the cytoplasm in the immediate vicinity of the central body is fine and dense. The late divisions with a large number of nuclei show conspicuous asters.

At the close of the last division the nuclei are beaked with the center at the tip of the beak and the astral rays radiating outward in all directions. The divisions take place near the wall and the centers ultimately appear to be in contact with the plasma membrane of the ascus.

The appearance of the distinct beak on the nucleus at the close of so many successive divisions is significant. In the few-spored ascus the beaked nuclei are conspicuous at the close of the third division. The beaks are unmistakable even in the very small nuclei in *Philocopra coeruleotecta*. The central body against the plasma membrane, with the clear-staining nuclear contents, and the relatively long thin beak, are all distinct in appearance.

The beaked nuclei with the centers against the ascus membrane, together with the strongly developed astral rays suggests at once that the delimitation in this many-spored form agrees with that found in the few-spored Ascomycetes. The interastral zones where the rays from adjacent asters meet is further evidence in this direction. Besides, some of the delicate fibers are visible as they turn downward over the nucleus. The long duration of the connection between the center and the spore membrane leaves no question that the spores are delimited by the astral rays as in the few-spored Ascomycetes.

A comparison between the processes here and those in *Geoglossum glabrum* shows them to be remarkably similar in the two cases. The beaked nuclei with their centers in contact with the ascus membrane, the conspicuous asters, the interastral zones, the turning downward of the tips of the newly delimited spores while the centers with the beaked nuclei still remain in contact with the spore membrane, all point toward a similar method of spore delimitation. This method was first described by Harper and is common in the few-spored forms of the Ascomycetes.

Lewis (19) described spore formation in *Pleurago zygospora*, a sixteen-spored Ascomycete. The delimitation occurs at the close of the third division, the same as in the eight-spored forms. A subsequent division produces the sixteen spores. The larger number of nuclei formed in *Philocopra coeruleotecta* before delimitation brought forth the question whether the method of delimitation is different from that described for the other Ascomycetes. Whether or not this method of delimitation is common to the other many-spored Ascomycetes is not yet determined. But the studies of Lewis (19) and Overton (22), as well as the present investigation, suggest that delimitation takes place in the same manner in all of the Ascomycetes. In some cases division may increase the number of spores. In other instances the nuclei may divide many times before delimitation takes place.

The ascus can in no way be compared with the sporangium in the Phycomycetes. It is probable that further work would reveal still greater uniformity in the process of spore formation in the Ascomycetes. Faull's assumption that the spores are delimited by the method found in the sporangia of the Phycomycetes finds no support in the many-spored Philocopra, where the process has been followed in detail.

There is apparently a direct relation between the nuclear divisions in the ascus and the position of the spores. The first division is either crosswise of the ascus or obliquely so, thus bringing the two resulting daughter nuclei nearer the ascus membrane. The succeeding divisions take place near the periphery of the ascus. The later divisions are nearer the membrane. At the close of the division period the beaked nuclei with the central bodies come in contact with the ascus membrane and the spores are delimited.

The important rôle played by the center and the nucleus in spore formation is apparent from their location near the region of greater activity. At the time of delimitation the nucleus and center are in contact with the ascus membrane. They are in contact with the spore membrane while the spore turns so that they point downward and outward. As the spore elongates they move back from the membrane to the opposite end, which increases in size and becomes the main body of the spore. The constant presence of the nucleus, with the central body, at the point where the main changes occur, suggests that they play more than a passive rôle in spore formation.

SUMMARY

1. The study of a many-spored Ascomycete is important for the determination of the true nature of the ascus. *Philocopra coeruleotecta* Rehm, a new species, is well suited for the study because the spores are comparatively large and the asci are found at various stages in the same pyrenocarp.

2. The cytoplasm in the ascus increases to a considerable extent before nuclear division begins.

3. The first division spindle lies crosswise of the ascus. This

brings the daughter nuclei nearer the ascus membrane. The nuclei in the following stages are near the periphery of the ascus.

4. Successive nuclear divisions follow one another rapidly until one hundred and twenty-eight nuclei are formed. The increase in number of nuclei is accompanied by a decrease in size.

5. At the close of the last division the nuclei are beaked. They are peripherally arranged in the central part of the ascus. The interior of the central part, the proximal portion, and the distal portion of the ascus contain cytoplasm devoid of nuclei.

6. The beaked nuclei, with the central bodies, come in contact with the plasma membrane.

7. So far as observable spore delimitation occurs by the bending back of the astral rays and their subsequent fusion to form the spore membrane.

8. The central body is present at all stages from the young ascus to the mature spore.

9. The delimitation of the spores separates the cytoplasm into the spore-plasm, which is the dense part immediately surrounding the nuclei, and the epiplasm, which fills the remaining part of the ascus.

10. At the time of delimitation the spores are ovoid in shape. The beaked nucleus and central body point toward the ascus membrane. Soon they turn around so that the central body and beaked nucleus point downward and outward. The spores elongate and grow obliquely upward toward the center of the ascus.

11. The central body and the nucleus are always in close relation to the region of greatest activity. After delimitation when the spores elongate, they leave the spore membrane and move toward the opposite end of the spore. The latter grows and becomes the main part of the spore. It is spherical in shape. The lower part remains long and slender and forms the tail of the spore.

12. A thick wall is laid down around the outside of the spherical portion of the spore. This is laid down by the spore-plasm. The epiplasm could not have played any rôle in the process since the wall between the tail portion and the main body is at least as thick as that of the rest of the spore wall. Yet the epiplasm was not in contact with the spore at this point.

13. There is no indication of any phylogenetic relationship between the ascus and the sporangium of the Phycomycetes. The many-

spored ascus does not show a process of spore formation intermediate between that of the sporangium and the ascus. The spores are delimited by the fusion of the astral rays as in the few-spored forms of the Ascomycetes.

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EXPLANATION OF PLATES IX–XI

The accompanying figures were made with the aid of a camera lucida. The magnifications are as follows:

Figure 1; 115 diameters.
Figures 2, 5, 6, 8, 10, 12, 14–16, 18, 19, 21; 1250 diameters.
Figures 3, 4, 7, 9, 11, 13, 17, 20; 1400 diameters.

PLATE IX

FIG. 1. Longitudinal section through pyrenocarp showing three asci and paraphyses.

FIG. 2. Very young ascus with primary nucleus.

FIG. 3. Young ascus, elongated.

FIG. 4. First division figure of primary nucleus of the ascus.

FIG. 5. Ascus cut obliquely. Four-nucleate stage.

FIG. 6. Portion of longitudinal section of ascus showing one of the spindle figures of the third division.

FIG. 7. Eight-nucleate stage. Three nuclei in the section drawn.

PLATE X

FIG. 8. Dividing figure of fourth-nucleate stage. Well-developed asters are: present.

FIG. 9. Section of ascus in 16-nucleate stage.

FIG. 10. Dividing figure from many-nucleate ascus.

FIG. 11. Longitudinal section through ascus after last division. Nuclei beaked and peripherally arranged with the centers toward the ascus membrane.

FIG. 12. Cross-section of ascus after the last division. Nuclear beaks directed outward.

FIG. 13. Oblique longitudinal section showing the interastral zones.

FIG. 14. Cross-section of ascus. Spores delimited.

FIG. 15. Longitudinal section of ascus with delimited spores. Nuclear beaks, with the centers, point downward and outward. Epiplasm vacuolate.

PLATE XI

FIG. 16. Spores elongated. Center and nucleus no longer in contact with the spore membrane.

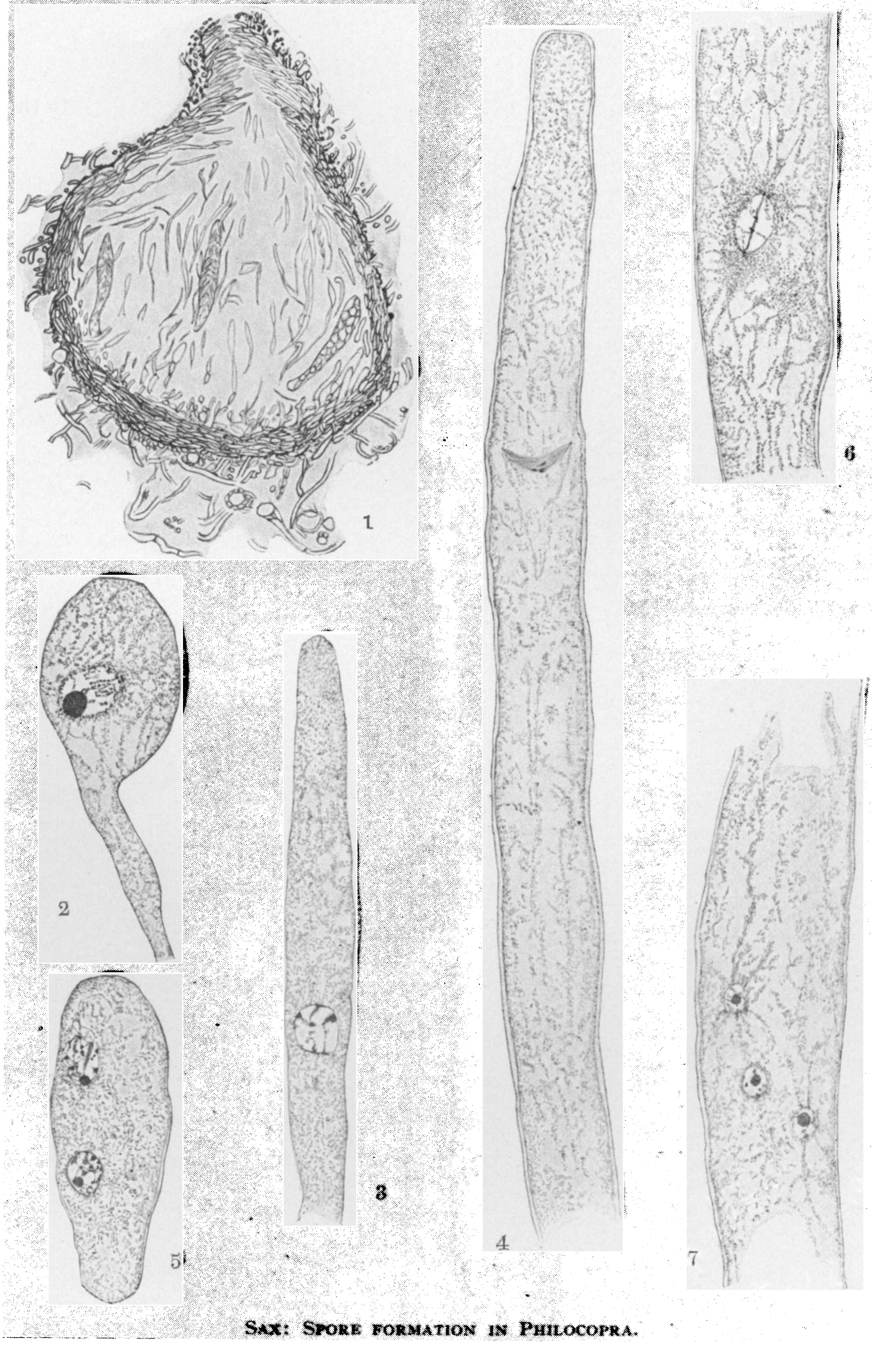
FIG. 17. Nucleus near the center of the spore.

FIG. 18. Spores larger at the upper end. Nuclei in or near the upper portion.

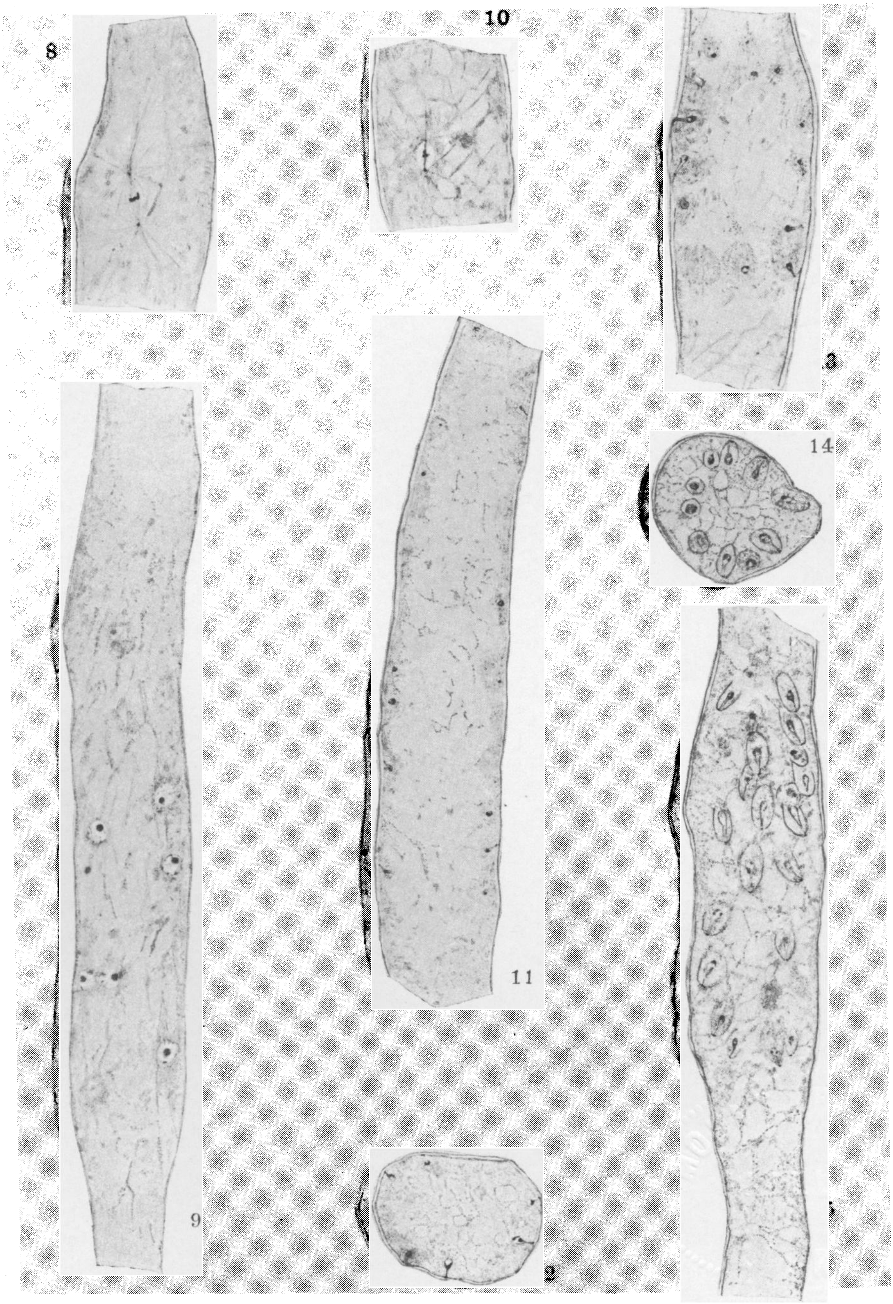
FIG. 19. Slightly older stage. Epiplasm distinct.

FIG. 20. Spores with large upper part. Nuclei in center of the spherical portions of the spores.

FIG. 21. Spores mature. Spherical portion enclosed by a thick wall. Tails hyaline.



SAX: SPORE FORMATION IN PHILOCOPRA.



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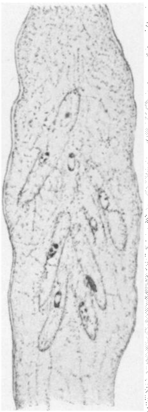

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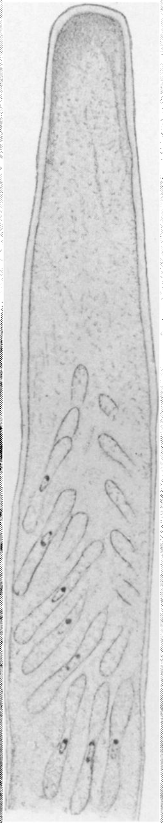

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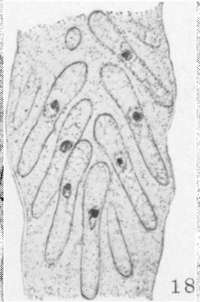

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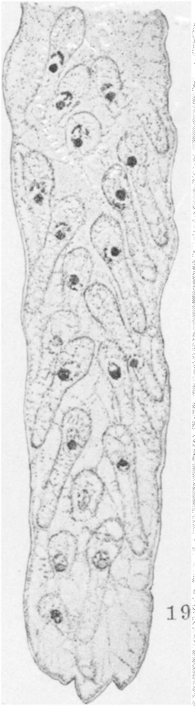

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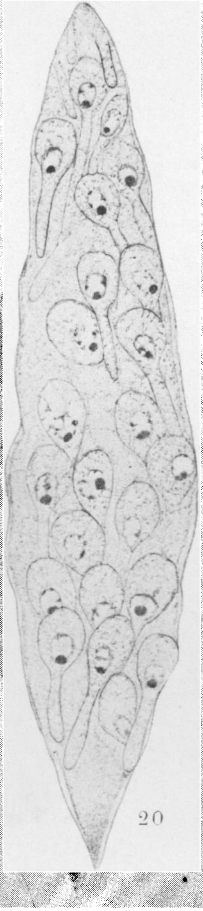

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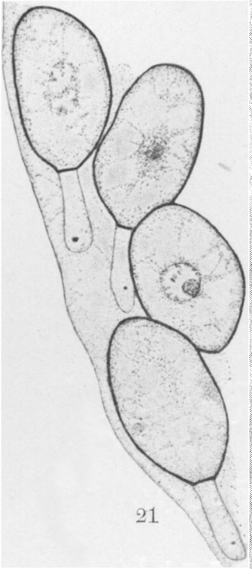

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SAX: SPORE FORMATION IN PHILOCOPRA.